

# A 37-kb restriction map of the human immunoglobulin lambda variable locus, VB cluster, harboring four functional genes and two non-coding $V\lambda$ sequences

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## ABSTRACT

The human immunoglobulin lambda variable locus (IGLV) is mapped at chromosome 22 band q11.1-q11.2. The 30 functional germline v-lambda genes sequenced until now have been subgrouped into 10 families ( $V\lambda 1$  to  $V\lambda 10$ ). The number of  $V\lambda$  genes has been estimated at approximately 70. This locus is formed by three gene clusters (VA, VB and VC) that encompass the variable coding genes (V) responsible for the synthesis of lambda-type Ig light chains, and the  $J\lambda$ -C $\lambda$  cluster with the joining segments and the constant genes. Recently the entire variable lambda gene locus was mapped by contig methodology and its one-megabase DNA totally sequenced. All the known functional V-lambda genes and pseudogenes were located. We screened a human genomic DNA cosmid library and isolated a clone with an insert of 37 kb (cosmid 8.3) encompassing four functional genes (IGLV7S1, IGLV1S1, IGLV1S2 and IGLV5a), a pseudogene ( $V\lambda A$ ) and a vestigial sequence (vg1) to study in detail the positions of the restriction sites surrounding the  $V\lambda$  genes. We generated a high resolution restriction map, locating 31 restriction sites in 37 kb of the VB cluster, a region rich in functional  $V\lambda$  genes. This mapping information opens the perspective for further RFLP studies and sequencing.

## INTRODUCTION

In response to the presence of foreign molecules, antigens, the B cells of vertebrates produce immunoglobulins (Ig or antibodies) that neutralize these antigens based on the principle of complementarity. Structurally, the immunoglobulins are formed by two identical heavy chains (55 kd each) and two identical

light chains (25 kd each) held together by disulfide bonds assuming a Y shape. Each heavy and light chain has a constant (C) region at the carboxy end and a variable (V) region at the amino end (Padlan, 1994).

Differences in the amino acid sequences of the heavy chain C regions in the base of the Y generate five immunoglobulin classes (IgG, IgM, IgA, IgE and IgD), but there are only two types of light chains, kappa ( $\kappa$ ) and lambda ( $\lambda$ ). Binding with the antigens occurs at the complementary determining regions (CDR 1, 2 and 3) located at the level of the V regions of each heavy and light chain (Kabat *et al.*, 1991).

The generation of diversity at the V regions is assured by three mechanisms: i) an inherited repertoire of germline V genes, ii) somatic recombination of V genes with diversity (D), junctional (J) segments and iii) somatic mutations of rearranged V-J or V-D-J genes

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(Tonegawa, 1983). In man, 40% of the serum antibodies are of the  $\lambda$  type, indicating the important role played by the  $\lambda$  chains in the antibody response (Lai *et al.*, 1989). The human IGLV locus is mapped at chromosome 22 band q11.1-q11.2 (McBride *et al.*, 1982; de la Chapelle *et al.*, 1983) and, based on the Southern hybridization studies, the number of the V $\lambda$  genes has been estimated at approximately 70 (Lai *et al.*, 1989).

The V $\lambda$  germline sequences described so far comprise 10 pseudogenes (Heiter *et al.*, 1981; Dariavach *et al.*, 1987; Alexandre *et al.*, 1989; Bauer and Blomberg, 1991; Combriato and Klobeck, 1991; Daley *et al.*, 1992a,b; Vasicek and Leder, 1990), three vestigial sequences (Frippiat and Lefranc, 1994; Williams *et al.*, 1996), one germline sequence (V $\lambda$ N.2) not assigned to any subgroup (Combriato and Klobeck, 1991) and 37 sequences with open-reading frame (ORF), 30 of which are functional: five V $\lambda$ I (Alexandre *et al.*, 1989; Brockly *et al.*, 1989; Siminovitch *et al.*, 1989; Bernard *et al.*, 1990; Daley *et al.*, 1992a; Williams and Winter, 1993), five V $\lambda$ II (Brockly *et al.*, 1989; Frippiat *et al.*, 1990; Williams and Winter, 1993), eight V $\lambda$ III (Combriato and Klobeck, 1991; Daley *et al.*, 1992b; Williams and Winter, 1993), three V $\lambda$ IV, three V $\lambda$ V, one V $\lambda$ VI (Williams and Winter, 1993), two V $\lambda$ VII (Alexandre *et al.*, 1989; Brockly *et al.*, 1989; Winkler *et al.*, 1992), one V $\lambda$ VIII (Winkler *et al.*, 1992), one V $\lambda$ IX (Williams and Winter, 1993; Passos Jr. *et al.*, 1994a) and one V $\lambda$ X (Stiernholm *et al.*, 1994). The complex structure of the human immunoglobulin lambda variable locus (IGLV) was elucidated by means of contig methodology using overlapping YAC and cosmid clones; the V $\lambda$  genes are arranged into three clusters (VA, VB and VC) that place the variable region coding genes within about an 800-kb region (Frippiat *et al.*, 1995; Kawasaki *et al.*, 1995). The physical map of the IGLV locus is available via Internet: <http://imgt.cnusc.fr.8104> or [www.ebi.ac.uk/imgt](http://www.ebi.ac.uk/imgt).

However, despite their importance for the humoral immune response and the above data, the molecular genetics of the  $\lambda$  chains is less known than that of heavy (H) and kappa ( $\kappa$ ) chains. The data about genetic polymorphism and recombination and the possible role of the IGLV locus in auto-immune or lympho-proliferative diseases are still incomplete.

To further analyze the positions of the restriction sites surrounding the V $\lambda$  genes of a region of the VB cluster we constructed a 37-kb detailed restriction map of a cosmid clone (cosmid 8.3; Passos *et al.*, 1994a,b,c; Frippiat *et al.*, 1995) that encompasses six immunoglobulin V $\lambda$  sequences, including four functional genes (IGLV1S1 (Alexandre *et al.*, 1989), IGLV1S2 (Bernard *et al.*, 1990), IGLV7S1 (Daley *et al.*, 1992a,b) and IGLV5a (Berinstein *et al.*, 1988)), and two

non-coding sequences (the pseudogene V $\lambda$ A and the vestigial sequence vg1; Alexandre *et al.*, 1989).

The detailed restriction mapping data of the cosmid 8.3 presented here represent a reference for further studies on RFLP of normal human populations and patients with auto-immune or lympho-proliferative diseases.

## MATERIAL AND METHODS

### Isolation and characterization of the cosmid 8.3

The human genomic cosmid library was kindly provided by Dr. L. Buluwela. It was prepared in the Loris-6 cosmid vector ligating *Hind*III DNA fragments of about 50 kb from the human Colo 320 cells to the *Hind*III cleaved vector (Buluwela *et al.*, 1988).

This library was screened with the IGLV1S1 gene probe (Alexandre *et al.*, 1989) and several positive clones were found. One of these clones, named cosmid 8.3, was amplified and its DNA was prepared using Qiagen purification columns (Diagen, Hilden, Germany) and digested with different restriction endonucleases (Boehringer, Mannheim). The restriction fragments were electrophoresed in 1% agarose gel and transferred to nylon membranes (Hybond N+, Amersham) according to standard procedures (Sambrook *et al.*, 1989). The filters were hybridized with the <sup>32</sup>P-labeled V $\lambda$  gene probes described below and washed under low stringency conditions. The probes used in the hybridizations were: the 5' IGLV1S1 insert probe, pV $\lambda$ 1VD0.2, which is a 216-bp *Pvu*II-*Hind*III fragment isolated from clone  $\lambda$ LY67V $\lambda$ I (Alexandre *et al.*, 1989) cloned in pUC-18, that contains the 5' region of the germline IGLV1S1 gene; the IGLV1S2 insert gene probe, pV $\lambda$ 2EK0.3, which is a 300-bp *Hinc*II-*Kpn*I fragment containing part of the IGLV2S2 gene cloned in pUC-18, and the IGLV7S1 insert gene probe, pV $\lambda$ 7BL0.68, which is a 680-bp *Bam*HI-*Bgl*III fragment isolated from pV3.3, a subclone of V4A (Daley *et al.*, 1992b) and subcloned in pUC-12. The pseudogene V $\lambda$ A (Alexandre *et al.*, 1989) and the gene IGLV5a (Berinstein *et al.*, 1988) were identified by PCR as described below.

### Polymerase chain reaction (PCR)

The cosmid 8.3 DNA was dissolved in 1x TE buffer (10 mM Tris-HCl, pH 7.5, with 1 mM EDTA) at a concentration of 100 ng/ $\mu$ l. The primers for the V $\lambda$ A pseudogene were 19-mer 5' TAATCCGTGTGCT-CAGGAA 3' forward and 18-mer 5' GGAGTGATCA-

GCCTTGTC 3' reverse (Chuchana *et al.*, 1991) and the primers for the IGLV5a gene were 37-mer 5' GGAA-TTCAAGCTTCTGCAGATGGCCTGGACTCCTCTC 3' forward and 25-mer 5' GGAATTCGCTTT-CTGTCTCACTTCC 3' reverse (Berinstein *et al.*, 1988).

The PCR amplifications were performed in a final volume of 30  $\mu$ l with 100 ng of cosmid 8.3 DNA, 40 pmol of each primer, 200  $\mu$ M of dNTPs and 2.5 units of Taq polymerase (Perkin Elmer Cetus, Norwalk, CT) in 100  $\mu$ l of 1x PCR buffer (200 mM Tris-HCl, pH 8.3, at room temperature with 1.5 mM MgCl<sub>2</sub>).

PCR profile: 94°C for 3 min, 55°C for 10 min. Thirty cycles each at: 72°C for 30 s, 94°C for 30 s, 55°C for 30 s, 72°C for 10 min. The PCR products were analyzed by 2% agarose gel electrophoresis.

### Subcloning fragments of the cosmid 8.3

The cosmid 8.3 DNA was digested separately with *EcoRI* and *HindIII*. The fragments were separated by electrophoresis in 1% low-melting point agarose (Sea-Plaque GTG, FMC, USA) in 1x TAE buffer (40 mM Tris-acetate, pH 8.0, plus 1 mM EDTA). After electrophoresis the gel was stained with a diluted ethidium bromide solution and each DNA band trimmed from the gel under short time UV exposition. The blocks of agarose containing DNA fragments up to 8.0 kb were melted at 68°C for 10 min and placed at 37°C to prevent solidification.

The DNA fragments were ligated to pUC-18 or pT7T318U vectors cleaved with *EcoRI* or *HindIII* in an in-gel ligation reaction (1  $\mu$ l of melted agarose containing the DNA fragment, 6.0  $\mu$ l of deionized and autoclaved water, 1.0 unit (1.0  $\mu$ l) of T4 DNA ligase (Boehringer Mannheim), 1.0  $\mu$ l (20 ng) of vector and 1.0  $\mu$ l of 10x ligation buffer. The mixture was left to stand at room temperature (25°C), for 18 h). The ligations were transfected to *E. coli* TG1 (Hanahan, 1983) and plated onto X-gal + IPTG-containing medium. The white clones harboring the correct size insert were identified by plasmid mini-preparations and digestion with the corresponding restriction enzyme (Sambrook *et al.*, 1989). Each positive clone was named according to the origin of the DNA, the restriction enzyme and the size of the insert.

### Restriction mapping

The plasmid mini-preparations of each cosmid subclone were digested with the *HindIII*, *EcoRI*, *BamHI*, *KpnI*, *XhoI* and *Sall* restriction enzymes (single and double digestions) and the fragments separated by electrophoresis in 0.8% agarose gel, 1x TBE buffer with

ethidium bromide. The gels were photographed under UV illumination and the molecular weight of each fragment was determined considering the presence of the vector.

The restriction fragments from the cosmid 8.3 exceeding 8.0 kb in size were not subcloned and the restriction data were obtained by direct in-gel digestion. The restriction map of cosmid 8.3 was assembled by overlapping these fragments and the maps of each subclone.

## RESULTS AND DISCUSSION

We identified several positive clones by screening the human genomic cosmid library from Colo 320 cells DNA (Buluwela *et al.*, 1988) with a specific V $\lambda$  gene probe insert (IGLV1S1 gene probe, Alexandre *et al.*, 1989), and from those presenting the strongest hybridization signal we selected the cosmid clone named 8.3 for further analysis. The digestion of the DNA from this cosmid with *EcoRI* and *HindIII* restriction enzymes permitted us to measure an insert of approximately 37.0 kb (Figure 1).

The Southern hybridization and PCR results with specific V $\lambda$  gene probes and primers demonstrate that this insert encompasses the functional V $\lambda$  genes IGLV1S1 (Alexandre *et al.*, 1989), IGLV1S2 (Bernard *et al.*, 1990), IGLV5a (Berinstein *et al.*, 1988) and IGLV7S1 (Daley *et al.*, 1992b), the pseudogene V $\lambda$ A and the vestigial sequence vg1 (Alexandre *et al.*, 1989) (data not shown).

We digested the cosmid 8.3 DNA separately with *EcoRI* and with *HindIII* in order to construct a precise map locating the restriction sites surrounding the six V $\lambda$  gene sequences. The *EcoRI* digestion produced fifteen 8.0- to 0.3-kb fragments. The 1.8-, 1.6-, 1.2- and 0.3-kb fragments corresponded to the Lorist-6 vector. *HindIII* digestion produced nine 16.0- to 1.0-kb fragments, and the entire Lorist-6 vector was liberated in a 5.0-kb fragment (Figure 1). The fragments below 8.0 kb were subcloned in pUC-18 or pT7T318U vectors (except for the Lorist-6 fragments).

We constructed the complete restriction map for the cosmid 8.3 by overlapping the restriction maps of each subclone (Figure 2). We located 31 restriction sites. The six V $\lambda$  gene sequences are dispersed among 22.8 kb in the 37.0-kb insert. The functional gene IGLV7S1 is 11.0 kb upstream from the IGLV1S1 gene.

The restriction map of the cosmid 8.3 overlaps with the map of phage clones  $\lambda$ LY67V $\lambda$ 1 (Alexandre *et al.*, 1989) and #4 (Daley *et al.*, 1992a) and YAC clones 366F5, 105H2 and 400B5 (Fripiat *et al.*, 1995),

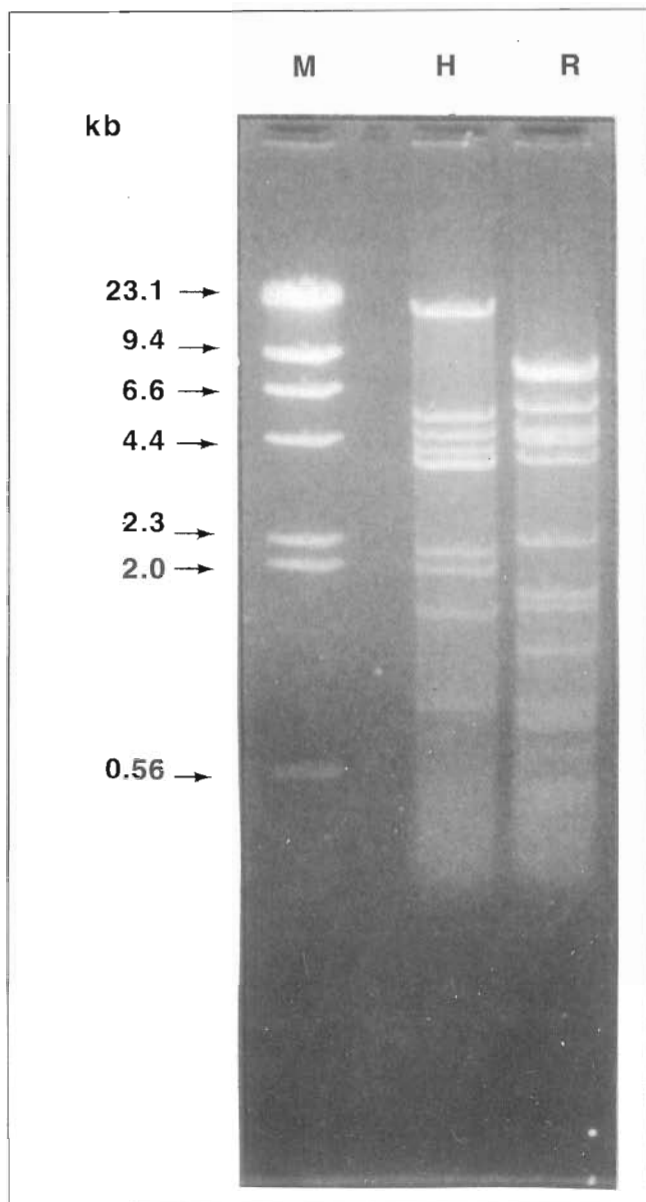


Figure 1 - Cosmid 8.3 DNA restriction fragments resolved on 1% agarose gel electrophoresis. M = Molecular weight marker fragments from  $\lambda$  phage DNA digested with *Hind*III. H = Cosmid 8.3 digested with *Hind*III. R = Cosmid 8.3 digested with *Eco*RI.

conferring a phage-cosmid-YAC contig. This confirms the position of the gene IGLV1S2 5.0 kb downstream of gene IGLV1S1. There is still a possibility that these genes evolved by duplication due to the sequence similarity (> 80%) and the positions of the restriction sites surrounding these genes. The two non-coding sequences (pseudogene V $\lambda$ A and vestigial sequence vg1) are located 2.0 kb and 7.0 kb, respectively, downstream of gene IGLV7S1 (Alexandre *et al.*, 1989; Chuchana *et al.*, 1993). Gene IGLV5a is located 4.3 kb downstream of the IGLV1S2 gene (Fripiat *et al.*, 1995).

Cosmid 8.3 is of particular interest since it covers a region of the cluster VB, IGLV locus, which is rich in functional V $\lambda$  genes and two non-coding

sequences (Figure 2, see cluster B). Several haplotypes were revealed by RFLP involving polymorphic insertion/deletion of genes IGLV1S1 and IGLV7S1 (Chuchana *et al.*, 1993) and IGLV5a (Fripiat *et al.*, 1995). Since these genes are present in cosmid 8.3, and with the more complete restriction map for this region and the collection of subclones of this cosmid, we can now do sequencing do perform RFLP studies of the normal human Brazilian population, and in patients with auto-immune or lympho-proliferative diseases.

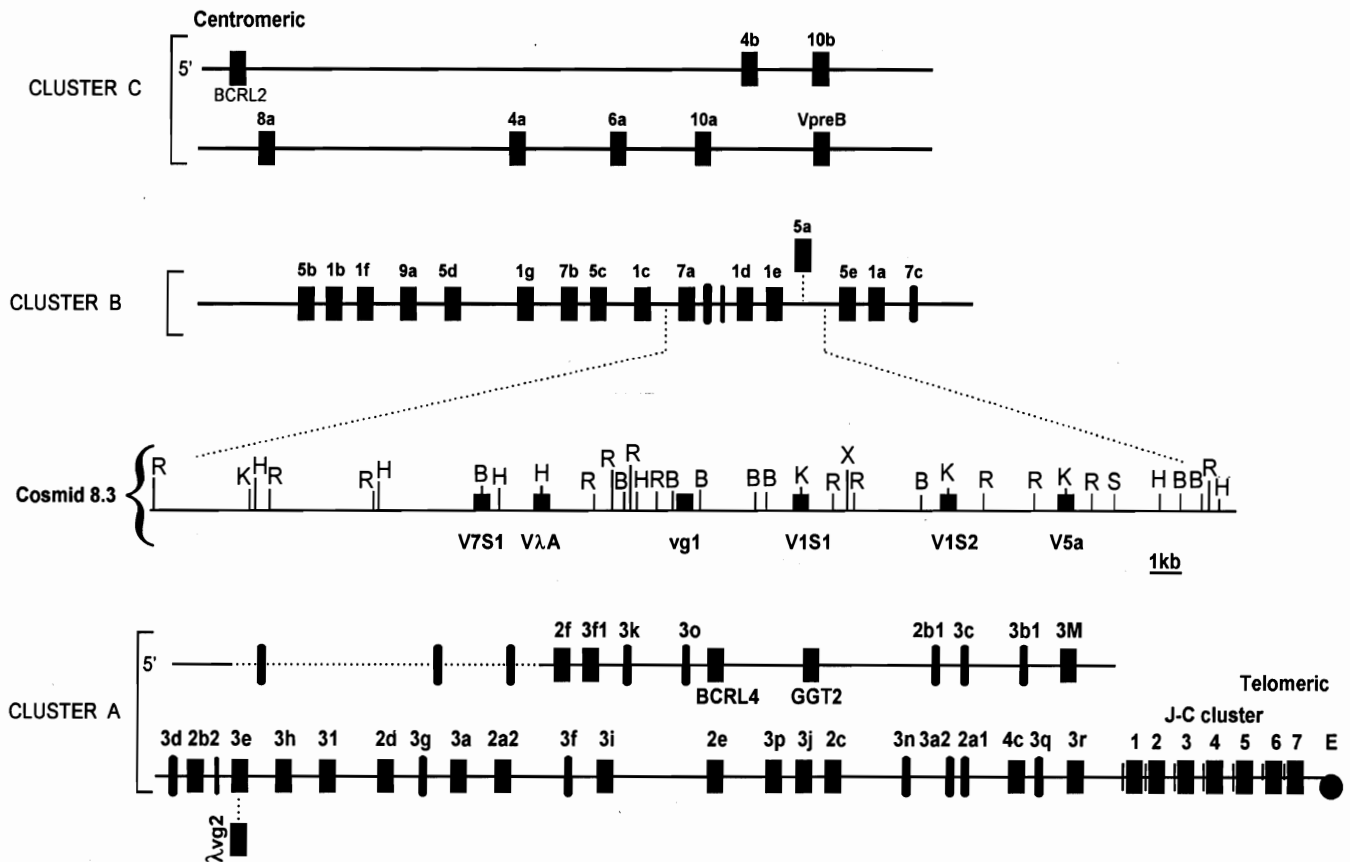
While this paper was under revision, Kawasaki *et al.* (1997) published one-megabase sequencing of IGLV locus representing the longest contiguous stretch of human DNA analyzed to date.

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## RESUMO

O locus lambda variável (IGLV) situa-se no cromossomo 22 banda q11.1-q11.2. Os 30 genes v-lambda germinativos e funcionais sequenciados até o momento foram distribuídos em 10 famílias (V $\lambda$ 1 a V $\lambda$ 10). O número de genes V $\lambda$  está próximo de 70. Este locus é formado por três grupamentos gênicos (VA, VB e VC) que contêm os genes codificadores das regiões variáveis (V) da cadeia leve tipo lambda e o grupamento J $\lambda$ -C $\lambda$  com os segmentos de junção (J) e genes das regiões constantes (C). Recentemente o locus IGLV foi mapeado (mapa físico) pela técnica dos "contigs" e seu um megabase de DNA totalmente seqüenciado, posicionando todos os genes V $\lambda$  funcionais e os pseudogenes. Neste trabalho, nós isolamos um clone de cosmídeo com 37 kb de inserto (cosmídeo 8.3) de uma biblioteca genômica humana preparada no vetor Lorist-6. Este cosmídeo contém quatro genes V $\lambda$  funcionais (IGLV7S1, IGLV1S1, IGLV1S2 e IGLV5a), um pseudogene (V $\lambda$ A) e uma seqüência vestigial (vg1) e nos permitiu estudar com detalhe as posições de sítios de restrição em torno dos genes V $\lambda$ . Geramos um mapa de restrição de alta resolução, localizando 31 sítios de restrição no grupamento genético VB, uma região rica em genes V $\lambda$  funcionais. Estas



**Figure 2** - Restriction map of cosmid 8.3 and its location in the cluster B of the IGL locus, chromosome 22q11.1-q11.2. The entire IGL locus spans about 800 kb and the positions of the V $\lambda$  genes in the clusters are not to scales (the 1-kb scale is valid only for cosmid 8.3). V $\lambda$  genes: V7S1 = IGLV7a (acc X14614), V1S1 = IGLV1d (acc M94118), V1S2 = IGLV1e (acc M94116), V5a = IGLV5a (acc Z73668), V $\lambda$ A = V $\lambda$ A pseudogene, vg1 = vg1 vestigial sequence. Sequences not related to the V $\lambda$  genes: BCRL2 and BCRL4 = BCR pseudogenes, VpreB = non Ig-like, GGT2 =  $\gamma$ -glutamyl transpeptidase-like sequence. This figure was based on the IMGT data base available via Internet: <http://imgt.cnusc.fr:8104> or [www.ebi.ac.uk/imgt](http://www.ebi.ac.uk/imgt)

informações sobre mapeamento abrem a perspectiva para estudos sobre RFLP e seqüenciamento.

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